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13. ABSTRACT (Maximum 200 words) The research assessed the fundamental character of the binding of polycyclic aromatic hydrocarbons (PAH) compounds to sediments that make the PAHs more or less bioavailable and toxic. Complementary techniques were employed to provide direct microscale information on the sequestration of PAH contaminants in Milwaukee Harbor sediment particles. Two-step laser mass spectrometry was used for PAH measurements, infrared microscopy was used for organic carbon measurements, and scanning electron microscopy with wavelength dispersive X-ray spectroscopy was used for elemental microanalysis. PAH concentrations on coal- and wood-derived particles were found to be several orders of magnitude higher than on silica particles. The coal/wood-derived particles constitute only 5% of the sediment by weight but contain 62% of the total PAHs. The remaining 38% are mainly in a clay and silt fraction. Desorption kinetic studies on these separated fractions revealed a relatively low availability of PAHs from the coal/wood fractions and a high availability from the clay/silt fractions. Preliminary work was done to understand the effects of degrading bacteria on contaminated sediment. The results indicated that bacteria and PAHs are co-located on the particles and degradation should not be hampered by spatial considerations.				
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Biostabilization of Polycyclic Aromatic Hydrocarbons (PAHs) Under Denitrification Conditions in Sediments

Statement of the Problem Studied

The research addressed the improved understanding of the fundamental character of the binding of polycyclic aromatic hydrocarbons (PAHs) at the microscopic scale, and examined how the nature of PAH association with sediments related to biostabilization, achievable treatment endpoints, toxicity and bioavailability. Owing to the abundance of polycyclic aromatic hydrocarbons (PAHs) in sediments and to the toxic, mutagenic, and carcinogenic effects attributed to these compounds, the cleanup of PAH-contaminated sediment has drawn increasing attention (1). Though bioremediation is a potentially cost-effective treatment for PAH-contaminated sediments, a fundamental long-term issue confronting sediment bioremediation is the lack of understanding of contaminant-sediment interactions and the impacts of such interactions on the failure to achieve treatment goals (1-4). Little is known about the mechanisms of PAH and other hydrophobic organic compound (HOC) sequestration and aging in soils and sediments and the resulting effect on chemical and biological availability (5). A major factor influencing successful sediment bioremediation is the availability of contaminants to microorganisms for degradation. However, contaminants that are strongly sorbed and not available to microorganisms may also not be available for a toxic response (6, 7). Thus, knowledge of how and where PAHs are bound to sediment material is necessary to assess the efficacy of sediment bioremediation and to correlate this knowledge with reductions in availability, mobility, and toxicity.

A major thrust of the proposed work was to explore the mechanisms controlling PAH sequestration using novel spectroscopic techniques to examine the distributions, associations, and binding energies of PAHs in sediments at the microscale. These data were used to correlate PAH associations with the locations and different forms of sorbent organic matter that are believed to play a significant role in the sequestration and bioavailability of PAHs on sediments. Previously, direct observational data revealing the

microscale location or locations in which nonpolar organic compounds accumulate when they associate with natural geosorbents did not exist. As a result, researchers relied on inferences from experimental tests of sorption for various sorbate/sorbent/solution cases of interest to develop an understanding of the physicochemical speciation of organic molecules in these solids. Certain macroscopic experimental observations can capture the overall behavior and provide empirical evidence for deducing sorbent/sorbate cases and constructing mechanistic models but are unable to provide direct information on microscale regimes. With the advent of increasingly sensitive instruments that offer higher spatial resolution, direct microscale observations have become possible. The work presented here involved the utilization of several microscale analytical techniques to examine microscale interactions of PAHs on sediments and soils.

Summary of work done & most important results

The sediment that was primarily used in this study was obtained from the Milwaukee Harbor Confined Disposal Facility (CDF) operated by the Milwaukee Harbor Port Authority. These sediments originated from the Milwaukee harbor during the process of dredging to maintain waterway navigability. Concerns have been raised about the potential for release of contaminants from such CDF sites and about closure requirements, as discussed by Bowman *et al.* (8). However, other contaminated sediments and soils have been investigated, and thus far there is reason to believe that the PAH binding patterns seen in Milwaukee Harbor sediment can be extrapolated to other sediments and soils.

In this research complementary microscale analytic techniques were employed to investigate where PAHs reside in sediments and to determine the material with which they are associated. A primary technique employed in this study was microprobe laser desorption/laser ionization mass spectrometry ($\mu\text{L}^2\text{MS}$), which measures PAH concentrations from a 40 μ diameter spot (9). This technique was supplemented with Fourier transform infrared (FTIR) microspectroscopy and scanning electron microscopy with wavelength dispersive X-ray spectroscopy (SEM/WDX) to evaluate the organic and elemental components in the sediment with which PAHs are associated. Figure 1 shows

the results of coincident microanalysis of elemental composition, organic carbon and microscale PAH analysis of Milwaukee sediment particles. A method for particle sectioning was developed that allowed the investigation of PAHs through the interior of sediment particles. The use of these novel instruments and techniques made it possible to describe the character of sorption mechanisms directly at a sub-particle level. Desorption kinetic tests were used to evaluate PAH affinity for the different sediment fractions identified in this study. The detailed analyses can be found in a recently published paper by Ghosh *et al.* (10).

The conclusions drawn from the work are:

1. Different locations on individual sediment and soil particles contain varying amounts of PAHs. The distances between the locations investigated are on the order of microns.
2. The surface concentration of PAHs can vary extensively depending on the type of particle. The majority of particles can be divided into two classes, coal-derived and silica.
3. Coal-derived particles contain the majority of PAH contamination (62%) but constitute only a small fraction (5%) of the whole sediment mass.
4. The PAHs on the coal-derived particles do not readily desorb in water. This observation may indicate that the PAHs will not leach out or be available to incite a toxic response from living organisms.
5. Silica surfaces contain few, if any, PAHs. It is possible that the <63 μm size fraction, in which PAH concentration is relatively high, contains silica particles. However, there is no indication that these particles would adsorb PAHs more readily than larger silica particles. Rather, the adsorbent in the <63 μm fraction is likely to be clay, which has been reported to have an affinity towards PAHs (11, 12).
6. The PAHs in the heavy, <63 μm clay/silt fraction are much more readily desorbed in water than the PAHs in the light, coal-derived fractions.
7. Contamination is confined to the near surface of particles. This observation indicates that PAH sequestration involves surface interactions rather than penetration into micropores of the particle.

Extending results to biodegradation studies

This work has established new methods for looking at contamination issues and has supplied direct measurements to evaluate past results. Part of the impetus for this work was to look for explanations as to why a fraction of PAHs is not available for degradation. Observations from numerous studies show that PAHs may be biodegraded by microorganisms to a residual concentration that no longer decreases with time, or that decreases very slowly, over years, with continued treatment (13). By directly identifying where PAHs reside in contaminated sediment and separating that sediment into differing components, PAH sequestration and availability is better understood. It is only one step, however, toward a final goal. The results have suggested the need for additional investigations. Several questions have been raised, including:

1. Where are degrading bacteria located on a particle? What kind of environment do they prefer?
2. Are the PAHs on coal-derived particles available to degrading bacteria?
3. Are the PAHs on the clay fraction available to degrading bacteria?
4. What is the toxicity of PAHs on coal-derived particles for living organisms?
5. What is the toxicity of PAHs on the clay fraction for living organisms?
6. Do the PAHs on the coal-derived particles result from processes occurring before their introduction into the harbor or are they acting as a sink for PAHs in the water?
7. Is separation by density a cost-effective method of clean up? On a small scale, as was used in this work, removal of the light component of the sediment is relatively simple, but this may not be the case when applied on a much larger scale.
8. Do these sediments need to be cleaned at all? It is possible that the coal-derived particles, which contain the majority of PAHs, bind them tightly enough so that they are unavailable to affect living organisms.

Currently, little is known about the microscale locations of degrading bacteria on contaminated sediment and soil. Work is underway to track both PAH concentration and bacteria location on sediment and soil particles. Figure 2 is a SEM picture of a Milwaukee sediment particle that was inoculated with PAH degrading bacteria. The particle pictured is a silica particle that has organic matter trapped in a depression. As

seen in Fig. 2, the bacteria are clustered around the depression containing the organic matter. Figure 3 shows the $\mu\text{L}^2\text{MS}$ spectra of two different spots on the particle. The bottom spectrum in the figure shows the PAH concentration from the area where the bacteria were congregated. This area also contains an abundance of PAHs. The top spectrum shows the PAH concentration from a bare silica spot that contains no organic matter or bacteria. These figures indicate that bacteria and PAHs are co-located on this sediment particle, and degradation should not be hampered by spatial considerations. It has not been determined whether the bacteria are attracted to the depression or the PAH food source.

Sample preparation for use of the SEM involves applying a light coating of gold on the particle's surface. This gold coating has made the desorption step used in $\mu\text{L}^2\text{MS}$ inconsistent and spectra are sometimes difficult to obtain. Ideally, no sample preparation or alteration would be needed to locate degrading bacteria on a particle's surface. Work is being done at the Naval Research Laboratory in Washington DC to genetically engineer microorganisms that produce a green fluorescent protein whose presence is made apparent when the bacteria are degrading PAHs. The genes for the initial oxidative attack on certain PAHs have been cloned and sequenced. It is therefore possible to develop a construct in which the gene for the production of GFP can be inserted into the initial part of the PAH degradation pathway. These organisms can then be added to sediment and soil particles containing sorbed PAHs and incubated. A spatial mapping of bacteria that are both degrading and not degrading PAHs on a particle's surface can be accomplished using an epifluorescent microscope. The particle will then be analyzed by $\mu\text{L}^2\text{MS}$, and PAH concentration can be correlated with bacteria location.

As stated before, it is clear that two major components are found in the whole sediment, a light component comprised of coal-derived particles and organic debris, and a heavy component comprised of silica particles and clay. Work is being accomplished to determine how each of these components reacts to biodegradation. At the Waterways Experiment Station (WES) in Vicksburg, MS, Milwaukee Harbor sediment has been placed into a series of bioslurry reactors containing PAH degrading bacteria. As the reactors operate, sub-samples of the sediment components are being removed and frozen at $-70\text{ }^\circ\text{C}$ for subsequent analysis using $\mu\text{L}^2\text{MS}$ and GC/MS. Additional analyses will

include thermal desorption mass spectrometry, PAH mineralization capacity, and the derivatization and detection of PAH degradation products. Preliminary results indicate that the PAHs contained in the light component are resistive to biodegradation, while those in the <63 μ m heavy component are degraded. These results are in agreement with previously completed desorption experiments. The different components of the sediment will also be tested at WES for toxicity to organisms. Preliminary tests are using earthworms as the bioassay system. More tests may include the freshwater Asiatic clam, *corbulica fluminea*. These investigations should provide useful information for determining clean-up procedures and endpoints.

The primary goal of the original proposal was to develop microscale characterization of PAH homolog distributions in sediments, and this has been successfully achieved. More direct evidence of PAH associations with geosorbents has been obtained. We had hoped to use the information to construct mechanistically-based conceptual models to aid interpretation of bioslurry denitrification treatment efficiency and toxicity of treated and untreated material from military and manufactured gas plants. Due to lack of funding in the second and third years, the denitrification processes could not be extensively examined.

List of all publications & technical reports:

None

List of all scientific personnel showing any advanced degrees earned by them while employed on the project:

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P. I. Professor Richard N. Zare

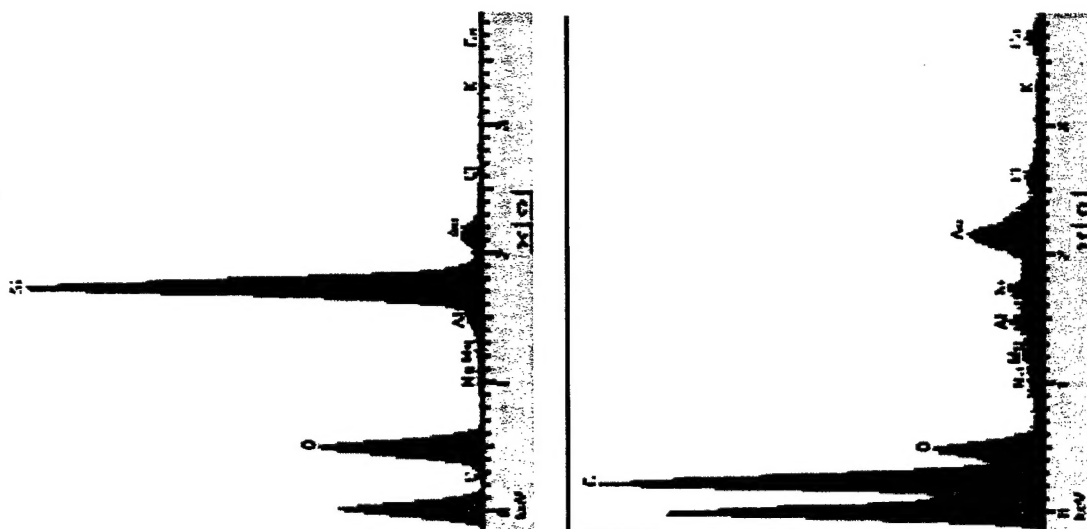
Report of Inventions:

None

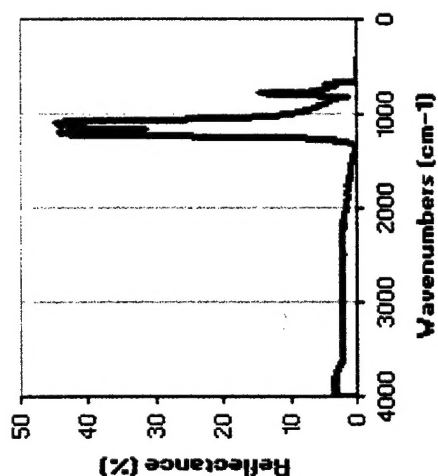
Bibliography

- (1) NRC *Contaminated Sediments in Ports and Waterways, Cleanup Strategies and Technologies*; National Research Council: Washington DC, 1997a.
- (2) Wilson, S. C.; Jones, K. C. *Environ. Pollut.* **1993**, *80*, 229-249.
- (3) NRC *Alternatives for Groundwater Cleanup*; National Research Council: Washington DC, 1994.
- (4) NRC *Innovations in Groundwater and Soil Cleanup*; National Research Council: Washington DC, 1997b.
- (5) Luthy, R. G.; Aiken, G. R.; Brusseau, M. L.; Cunningham, S. D.; Gschwend, P. M.; Pignatello, J. J.; Reinhard, M.; Traina, S.; Jr., W. J. W.; Westall, J. C. *Environ. Sci. Technol* **1997**, *31*, 3341-3347.
- (6) Linz, D. G.; Nakles, D. V. *Environmentally Acceptable Endpoints in Soil*; American Academy of Environmental Engineers: Anapolis, MD, 1997.
- (7) Tang, J.; Carroquino, M. J.; Robertson, B. K.; Alexander, M. *Environ. Sci. Technol.* **1998**, *32*, 3586-3590.
- (8) Bowman, D. W.; Brannon, J. M.; Batterman, S. A. *Proceedings of the 11th US Army Corps of Engineers Waterways Experiment Station Seminar*, 1996.
- (9) Gillette, J. S.; Luthy, R. G.; Clemett, S. J.; Zare, R. N. *Environ. Sci. Tech.* **1999**, *33*, 1185-1192.
- (10) Ghosh, U.; Gillette, J. S.; Luthy, R. G.; Zare, R. Z. *Environ. Sci. Technol.* **2000**, *34*, 1729-1736.
- (11) Burkow, I. C.; Jorgenson, E.; Meyer, T.; Rekdal, O.; Sydnes, L. K. *Org. Geochem.* **1990**, *15*, 101-108.
- (12) Karimi-Loftabad, S.; Pickard, M. A.; Gray, M. R. *Environ. Sci. Technol.* **1996**, *30*, 1145-1151.
- (13) Luthy, R. G.; Dzombak, D. A.; Peters, C. A.; Roy, S. B.; Ramaswami, A.; Nakles, D. V.; Nott, B. R. *Environ. Sci. Technol.* **1994**, *28*, 266A-277A.

Elemental Analysis



IR Analysis



PAH Analysis

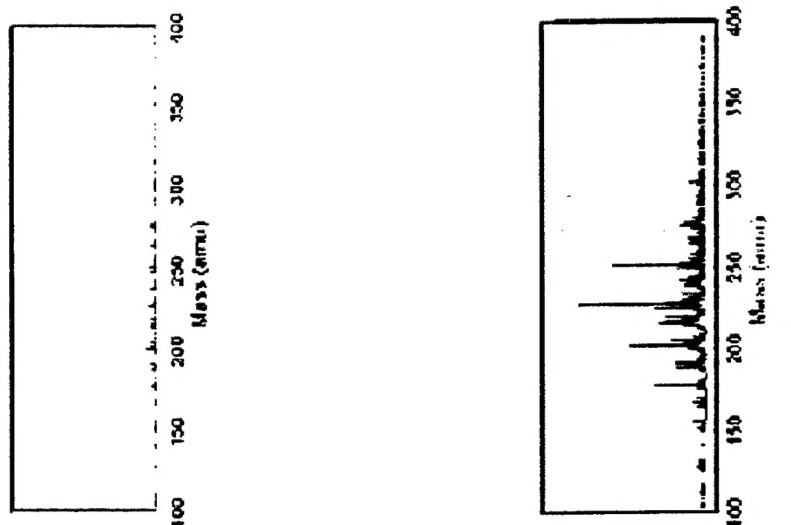


Figure 1. Analyses of elemental composition, organic carbon, and PAH distributions of Milwaukee sediment particles

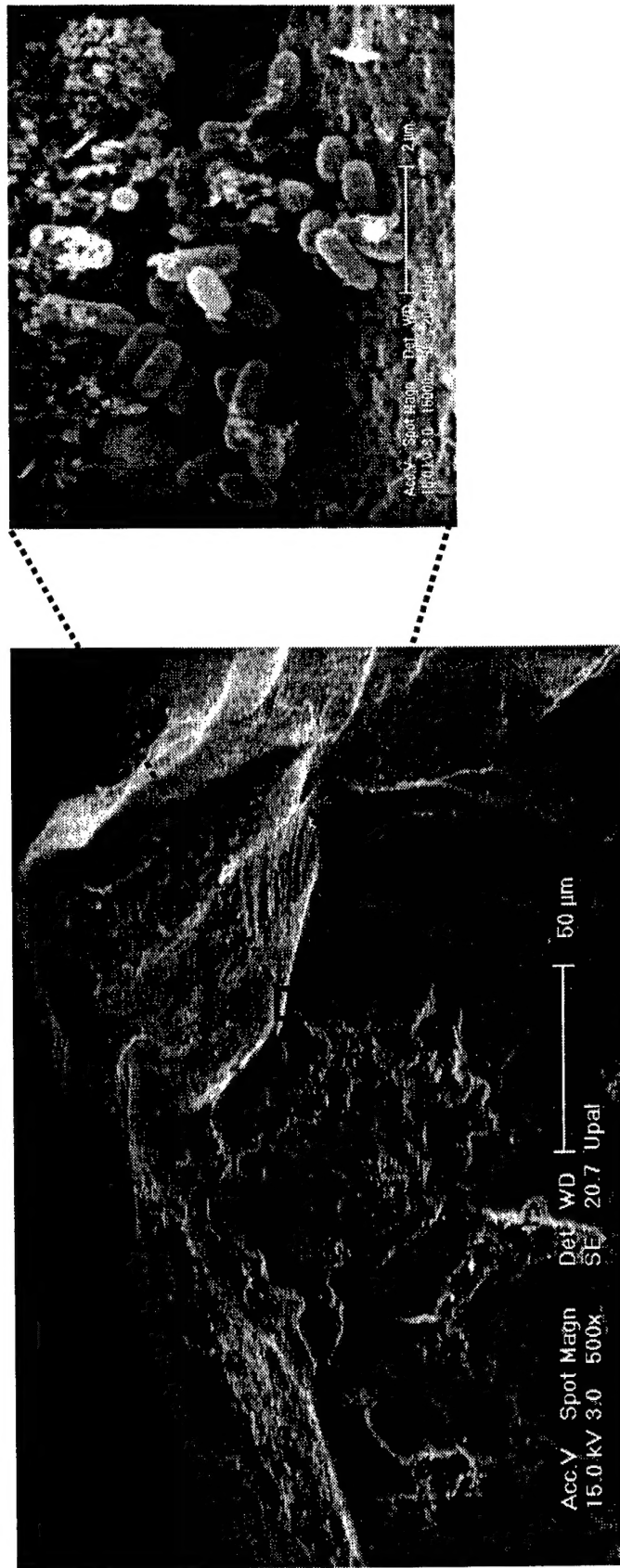


Figure 2. SEM pictures of a silica particle with attached organic matter (left) and bacteria that are clustered on a patch of attached organic matter (right).

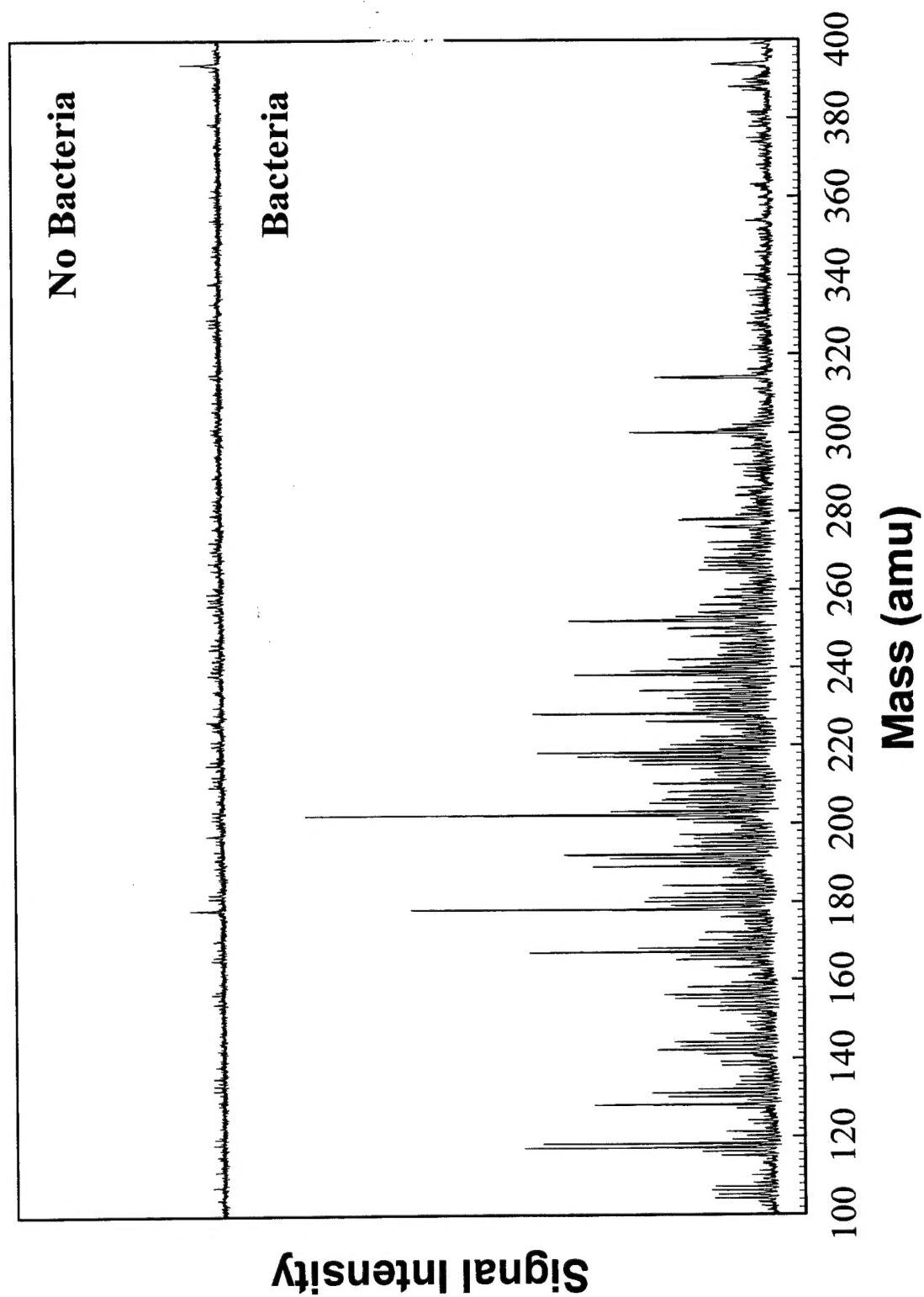


Figure 3. $\mu\text{L}^2\text{MS}$ spectra of silica particle pictured in Figure 2, with bacteria absent (top) and present (bottom).